ALGICIDES AND AQUATIC HERBICIDES

TASTE AND ODOUR IN WATER

LABORATORY STUDIES ON FISH TOXICITY

FIELD TOXICITY STUDIES

BY

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Biology Branch

Laboratory Division

ONTARIO WATER RESOURCES COMMISSION

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REPORT ON

ALGICIDES AND

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SUMMARY

Studies related to the use of algicides and aquatic herbicides have been made by personnel of the Biology Branch.

Determination of the intensity of taste and odour of seven representative herbicides in water indicated that considerable dilution of certain products is necessary to prevent tainting.

Data obtained on the acute toxicity to fish of 28 products showed that several provide adequate margins of safety for fish when used at suggested rates, while others are potentially lethal at low concentrations. Use of the latter should be limited and judicious.

Different formulations of the same active ingredient were found to vary in their fish toxicity and intensity of taste and odour.

A limited study of residual toxicity to fish indicated that toxicity may decrease with time and the rate of detoxification of certain chemicals is increased by biological activity.

Observations on lakes and ponds treated chemically supported results of laboratory studies of fish toxicity and showed that four chemicals did not produce acute toxic effects among aquatic invertebrate populations.

INTRODUCTION

Excessive growth of algae and other aquatic plants may render water unsuitable for domestic water supply, industrial use and recreational pursuits. Chemical control of these aquatic nuisances has increased considerably in recent years concurrent with a burgeoning utilization of ponds, lakes and streams. Where copper sulphate and sodium arsenite were once the only algicide and herbicide available, now industry has developed a great variety of products designated for use against algae and submerged, emergent and floating aquatic plants.

The introduction of these new chemicals to our waterways has raised questions in regard to possible detrimental side-effects. Not only aquatic fauna and wildfowl, but man himself could be exposed to toxic substances. Products registered for use in Canada have been subjected to lengthy mammalian toxicity tests to safeguard public health. The misuse of these or imported chemicals by the uninformed, however, would be potentially dangerous. The threat of indiscriminate use of aquatic herbicidal substances has been reduced greatly in Ontario by legislation of the Ontario Water Resources Commission enacted in 1962, whereby a permit is required for the addition of pesticidal chemicals to water.

The basis of the permit system is an intimate knowledge of not only the efficacy, but all undesirable characteristics of the chemicals proposed for application. Although information was available on the mammalian and fish toxicity of many products, it was deemed advisable that the OWRC investigate certain aspects related to the use of as many chemicals as possible. This report presents the results of laboratory studies on the taste and odour of herbicides in water, acute toxicity to native fish, residual fish toxicity, and field observations on the toxicity of certain chemicals to fish and other aquatic life.

The studies outlined were made during 1962 and early 1963 under the direction of John H. Neil, Supervisor of the Biology Branch. Laboratory investigations were conducted by Yvonne H. Swabey, and the field observations were made by Carl F. Schenk.

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The excellent co-operation received from Mr. M. G. Johnson of the Metropolitan Toronto and Region Conservation Authority and Mr. K. Irizawa, District Biologist, Department of Lands and Forests, Lindsay, Ontario, in the field studies related to the toxicity of herbicides, was very much appreciated. Several Conservation Officers of the Lindsay Forest District of the Department of Lands and Forests gave valued assistance also.

TASTE AND ODOUR

One factor to be considered in the application of aquatic herbicides is the possibility of taste and odour imparted to a domestic water supply even though the chemical is applied at a sufficient distance from the intake to preclude the possibility of toxicity. The intensity of taste and odour produced by seven representative products was investigated by a series of tests employing

a panel of observers. The object of these tests was to determine concentrations of the chemicals which yielded unanimous negative response by the panel. Products tested included five liquids: endothal, Aqualin, Hydrothol 47 and two formulations of silvex, Kurosal SL and Kuron. Two granular formulations of 2,4-D, Esteron 99 and Crop Rider, were tested also. The active ingredients of these and other products discussed in subsequent sections of this report are given in Table 1.

Methods and Procedures

A panel of six observers, chosen at random from the laboratory staff, reported daily at four times set at least one hour after meals. The observers were cautioned against the use of perfumed cosmetics, and each washed his hands with "Ivory" soap before tests. Tests were held in an unused air-conditioned room.

Odour- and taste-free water, produced by passing tap water through activated carbon and gravel, was used throughout the tests for preparation of solutions and rinsing of glassware. Stock and test solutions of liquid herbicides were prepared within the hour before each test. Concentrations of active ingredient in the stock solutions were as follows: endothal 5 ppm., Kurosal and Kuron 2 ppm., Aqualin 4 ppm. and Hydrothol 2.4 ppm. The granular formulations were applied to water at the rate of 0.1 gm. per 4 litres, or 5 mg. active per litre, which would be comparable to a field application of 100 lb. product per acre of water, 1.5 feet deep. These were allowed to leach for periods of three and twenty-four hours before the supernatant in each case was decanted for dilution.

Forty degrees Centigrade is the preferred temperature for taste tests since no sensation of hot or cold is detectable (A.P.H.A., 1960). Solutions for tasting were warmed to this temperature before they were dispensed to individual

Table 1. Active ingredients and their concentrations in products tested.

Product	Active Ingredient	Active Concentration
Amitrol T	Aminotriazole + ammonium thiocyanate	2 lb/Imp. gal.
Aqualin	Acrolein	85% by wt.
Aquathol-Silvex	3,6 - endoxo hexahydropthalic acid + 2-(2,4,5 - trichlorophenoxy) propionic acid	2 lb) 4 lb/U.S. gal. 2 lb)
Atlas A	Sodium arsenite	3.6 lb As/Imp. gal.
Atrazine (Pdr)*	2 - chloro - 4 - ethylamine - 6 - isopropylamino - s - triazine	50%
Copper sulphate	Copper	25%
Crop Rider (Gr)**	2,4 - dichlorophenoxyacetic acid, ethyl hexyl ester	20% 2,4-D
Endothal	3,6 - endoxo hexahydrophthalic acid	1.7 lb/Imp. gal.
Esteron 99 (Gr)	2,4-D, propylene glycol butyl ether esters	20% 2,4-D
Fenac	2,3,6 - trichlorophenylacetic acid	1.5 lb/U.S. gal.
Fenac (Gr)	" " "	10%
FW - 925	2,4 - dichlorophenyl 4-nitrophenyl ether	4 lb/U.S. gal.
Garlon	2,2 - dichloropropionic acid + 2-(2,4,5 - trichlorophenoxy) propionic acid	4.8 lb) 5.4 lb/ 0.6 lb) Imp. gal.
Hyamine 2389	alkyl (C_{14}, C_{12}, C_{16}) dimethyl benzyl ammonium chlorides	50%
Hydrothol 47	Endothal, di NN dimethyl cocoamine salt	1 lb/U.S. gal.
Kuron	Silvex, butyl ether esters	4.8 lb/Imp. gal.
Kurosal SL	Silvex, potassium salt	6 lb acid/U.S. gal.
Kurosal G.(Gr)	" " "	20% acid
NIA 5625	Tripropyltin chloride	50%

* Powder formulation. ** Granular formulation.

Continued.

Table 1 (Continued).

Product	Active Ingredient	Active Concentration
Penco 191	Endothal, mono NN dimethyl cocoamine salt	2 lb/U.S. gal.
Phygon XL (Pdr)	2,3 - dichloronaphthoquinone	50%
Reglone DB	1:1'- ethylene - 2:2'- dipyridylium dibromide	2 lb/Imp. gal.
Reglone DC	1:1'- ethylene - 2:2'- dipyridylium dichloride	2 lb/Imp. gal
Simazine (Pdr)	2, chloro - 4,6 - bis(ethylamino) - s - triazine	50%
Stam F-34	3,4 dichloropropionanilide	3 lb/U.S. gal.
Urab	3 - phenyl-1, 1 - dimethylurea trichloroacetate	3 lb/U.S. gal.
Urox	3(p - chlorophenyl) - 1, 1 - dimethyl-urea trichloroacetate	3 lb/U.S. gal.
Weed Rhap (Gr)	2,4-D, ethyl hexyl ester	20% 2,4-D

beakers. Four concentrations of herbicide and two blanks of taste-free water were presented to the panel members at one time. Taste-free water was provided for a mouth-wash between samples.

The standard temperature of 60° C., which facilitates odour detection, was used in the odour tests. Solutions were warmed to this temperature in stoppered flasks which were shaken and tested in turn by the observers. Here

again, four sample concentrations and two blanks were presented, and a flask of odour-free water was provided for reference.

Due to the volatile nature of Aqualin, additional tests were made on solutions at 21° C. to determine whether taste and odour were more intense at the lower temperature.

A wide range of concentrations was first presented to the panel and narrowed repeatedly thereafter. Results were recorded as the presence or absence of detectable taste or odour. Tests on each chemical were terminated when a concentration was found where no member of the panel recorded a positive response.

Results

Results of the tests are given in Table 2. Since the primary purpose of the study was to determine the concentrations at which no member of the panel could detect taste or odour, these data are given in the column headed "all negative".

Table 2. Taste and odour of certain herbicides in water at 40° C. and 60° C., respectively

Product	Stock Conc'n, ppm.	TASTE, Conc.(ppm)where majority negative	all negative	ODOUR, Conc.(ppm)where majority negative	all negative
Liquid:					
Endothal	5	2.5	2.5	1.25	1.25
Kurosal SL	2	2.0	2.0	.25	.25
Aqualin	4	.2	.1	.08	.07
Hydrothol 47	2.4	.2	.1	.006	.004
Kuron	2	.0047	.0044	.0029	.0027
Granular:					
Esteron 99 (3 hr.)	5	.17	.08	.25	.16
Esteron 99 (24 hr)	5	.41	.20	.50	.40
Crop Rider (3 hr.)	5	.01	.01	.05	.025
Crop Rider (24 hr)	5	.013	.01	.03	.02

Use of this criterion, however, may produce aberrations in the data due to one or two highly sensitive panelists. Cognizance is taken of this by listing also the concentrations at which the majority of the panel indicated negative reactions. All concentrations cited in the table and throughout the text are in terms of active ingredient of the products.

Discussion and Conclusions

Of the five liquids tested, endothal and Kurosal produced least taste and odour in water. Taste was not detected in the stock solution of 2 ppm. Kurosal, and the 5 ppm. solution of endothal required dilution by only an equal volume to negate taste. The odour of endothal was not detected at 1.25 ppm., nor that of Kurosal at 0.25 ppm. The taste and odour of Kuron were the most persistent, requiring dilution to 4.4 ppb. for taste and 2.7 ppb. for odour, before negative results were achieved. These concentrations represent dilutions of 450 and 740 times the stock solution of 2 ppm.

The data for Kuron and Kurosal indicate a great difference in the taste and odour characteristics of these two formulations of silvex. Similarly, there was a considerable difference between endothal and its cocoamine salt formulation in Hydrothol 47, which had to be diluted to 0.1 ppm. to prevent taste and to 0.004 ppm. to obviate odour.

At the standard test temperature the taste level of Aqualin was the same as that of Hydrothol 47, while its odour was weaker. Tests on Aqualin at 21° C. indicated no change in the odour level at the cooler temperature, but the taste was increased so that dilution to 0.02 ppm. was necessary for negative response.

All of the liquid herbicides exhibited stronger odours than tastes. In the case of the granular 2,4-D products the reverse was true. Of the two

tested, Esteron 99 had the lesser taste and odour, Crop Rider the greater. After a 24-hour leach, the latter product required dilution to .01 ppm. for negative taste response and to 0.02 ppm. for undetectable odour. Both the taste and odour of Esteron 99 decreased when the product was allowed to leach 24 hours rather than three. Those of Crop Rider, however, did not change appreciably over a similar period.

The data obtained in these tests indicate that a dilution of only eight times the commonly applied concentrations of endothal and Kurosal would be sufficient to negate taste and odour problems. For Aqualin, Hydrothol 47, Kuron, Esteron 99 and Crop Rider dilutions of 63 to 740 times would be required. Certainly the factors of taste and odour must be considered when the use of an algicide or herbicide in the vicinity of a water intake is proposed.

ACUTE TOXICITY TO FISH

Twenty-eight algicides and herbicides were tested for their acute toxicity to lake emerald shiners, Notropis atherinoides.

Methods and Procedures

From February to May, 1962, stocks of emerald shiners were obtained from a local bait dealer who had collected them from Lake Erie. These fish were maintained in running Toronto tap water at 55° F. until the last were used in May. In December several thousand fish of the same species were seined from Georgian Bay. These were held for tests made through March, 1963. While in the holding tanks the fish were fed daily with dogfood and/or frozen Daphnia or brine shrimp. They appeared to be healthy and little mortality occurred. Periodically, numbers of fish were transferred to five-gallon jars and allowed to acclimate to room temperature (71° F.) in the laboratory. They were held in tap

water for seven to 14 days and not fed for two days prior to tests. Fish were not used for bioassay if mortality in the four days preceding the test amounted to 10 per cent. Mortality during this period usually ranged from zero to three per cent.

Test procedures followed were those given in "Standard Methods" (A.P.H.A. et al, 1960) with the exception that five fish were exposed to each concentration rather than ten. Ten fish were held as controls.

All tests were run in Toronto tap water which had been standing at least one day in the laboratory. The tap water has an annual mean total hardness of 132 ppm. as $CaCO_3$ and a range of 130 to 135 ppm. The dilution water was aerated for one hour before preparation of test solutions and the solutions were not usually aerated thereafter. The exceptions were applications of the granular products Crop Rider and Kurosal G in which surface films inhibited absorption of oxygen from the air. In these instances only, a slow stream of large air bubbles was passed through for an hour at 48 and 72 hours.

The dissolved oxygen content of the solutions was determined at the beginning of the tests and after mortality. The pH range of the concentrations tested was recorded and water temperatures determined by a maximum-minimum thermometer.

Results

Data on the length and weight of fish used, solution temperature, pH and oxygen content are given in Table 3. Table 4 presents the median tolerance limits (TL_m), that is, the concentrations at which 50 per cent of the test fish survived, for four, 24, 48 and 96 hours. These data are arranged roughly in order of increasing toxicity. The TL_m's represent milligrams active ingredient per litre except for the Hyamines and NIA 5625 which are parts per million by

volume. Active concentrations of the products are given in Table 1.

It should be noted that the figures given for granular products were obtained on the basis of applied rates and that the actual quantities of active ingredient present in solution are not known.

Discussion and Conclusions

The 96-hour TL_m's obtained were from .03 to 510 ppm., indicating a wide range of toxicity among the products tested. Based on the order of magnitude of the TL_m's the chemicals fall into five groups. Relatively non-toxic, with TL_m's in excess of 250 ppm., were Aquathol-Silvex, Amitrol T, Kurosal G and SL (silvex, potassium salt), Crop Rider and Weed Rhap (2,4-D, ethyl hexyl ester). The granular formulation of Kurosal yielded a TL_m somewhat higher than the liquid product, due possibly to leaching rate.

The dibromide formulation of Reglone (diquat) was moderately toxic with a 96-hour TL_m of 25.8 ppm. The dichloride formulation of this chemical had a TL_m of 9.1, placing it in the next group with TL_m's between two and 10 ppm. The remainder of this group consisted of Atlas A, Esteron 99 (2,4-D, butyl ether esters), Garlon, Urab, Urox, Hyamine 2389 and Kuron (silvex, butyl ether esters).

Hyamine 3500 and Penco 191 were more toxic with TL_m's of 0.75 and 0.35 ppm., respectively. Five chemicals which exhibited TL_m's of less than 0.1 ppm. comprised the final group. Included were copper sulphate, Hydrothol 47, Phygon XL, Aqualin and NIA 5625. Most of these are effective algicides at very low concentrations.

It will be noted that median tolerance limits were not derived for Simazine. Since this product is reputed to be soluble only up to five ppm., testing was limited to applied concentrations of 5.6, 10.0 and 18.0 ppm. active. It was non-toxic at these rates.

Table 3. Toxicity test accessory data.

Product	Mean fish length, mm.	gm fish/litre	Mean temp. °F.	pH. Range.	DO Range ppm.
Amitrol T	53	0.5	70	4.7 - 6.9	5.62 - 6.36
Aqualin	75	0.7	70.5	7.7 - 8.1	5.62 - 7.82
Aquathol-Silvex	55	0.6	70	7.8 - 7.9	5.90 - 8.84
Atlas A	50	0.2	72	8.1 - 8.9	Indet.
Atrazine	57	0.6	71	8.1 - 8.2	5.02 - 8.00
Copper sulphate	60	0.7	70	8.0	5.36 - 8.64
Crop Rider	61	0.8	71.5	7.5 - 8.0	4.10 - 8.31
Esteron 99	64	0.7	72	7.8 - 8.2	5.46 - 8.67
Fenac	59	0.6	70	8.1 - 8.2	5.82 - 8.60
Fenac (Gr.)	59	0.6	70	8.1 - 8.2	5.36 - 8.96
FW-925	59	0.6	71	8.2 - 8.3	6.00 - 7.96
Garlon	61	0.6	71	7.7 - 8.2	6.62 - 8.72
Hyamine 2389	62	0.4	71	7.7 - 8.3	6.60 - 8.72
Hyamine 3500	60	0.6	71	8.1	6.10 - 8.40
Hydrothol 47	61	0.5	71	7.8 - 8.0	6.18 - 8.88
Kuron	46	0.2	72	7.8 - 8.3	6.26 - 8.80
Kurosal SL	62	0.5	71	7.9 - 8.2	6.98 - 8.56
Kurosal G (Gr.)	59	0.6	71.5	7.8 - 8.1	4.60 - 8.20
NIA 5625	53	0.5	70	8.0 - 8.3	6.10 - 7.30
Penco 191	61	0.7	70.5	8.2	5.32 - 8.38
Phygon XL	46	0.3	72	7.8 - 8.3	7.00 - 8.62
Reglone DB	55	0.6	70	7.7 - 8.0	Indet.
Reglone DC	55	0.6	70	7.6 - 8.0	Indet.
Simazine	55	0.5	70.5	8.3	4.94 - 8.16
Stam F-34	59	0.6	70	8.1 - 8.3	4.34 - 8.90
Urab	43	0.2	71	7.8 - 8.0	7.80 - 8.62
Urox	58	0.6	70	7.9 - 8.0	4.02 - 6.82

Simazine and Atrazine in tablet form were tested also. As these preparations are intended to sink into the mud, tests were run in jars containing 10 litres of water over two inches of river mud. The tablets, applied at the suggested rate of nine per square foot, fragmented on the mud surface but were non-toxic to fish exposed for 96 hours.

The data for Aqualin in Table 4 were obtained during residual toxicity tests reported in a subsequent section. Since the fish used in the residual tests appeared to be hypersensitive, these TL_m's may be slightly lower than would

have been obtained with other groups of fish. Aqualin was originally tested with common shiners, Notropis cornutus. Median tolerance limits derived for this species were 0.25 ppm. at four hours and 0.07 ppm. from 24 to 96 hours.

Table 4. Acute toxicity of algicides and herbicides to lake emerald shiners.

Product	Liquid Gran. Powder	TL_m, ppm. active 4 hr.	24 hr.	48 hr.	96 hr.
Aquathol-Silvex	L	>1000	780	612	510
Weed Rhap 20	Gr.	>1000	620	620	510
Amitrol T	L	910	455	455	420
Kurosal G	G	1100	540	450	370
Crop Rider	G	500	280	280	280
Kurosal SL	L	509	520	310	270
Reglone (DB)	L	>180	>180	86.2	25.8
Fenac	Gr.	>100	45.5	39.5	29.0
Fenac	L	>100	42.5	25.8	24.0
Atrazine	Pdr.	42.5	24.0	19.8	15.6
Reglone (DC)	L	>180	15.5	11.7	9.1
Atlas A	L	>32	13.5	8.1	8.1
Stam F-34	L	13.5	7.5	7.5	7.5
Esteron 99	Gr.	>10.0	4.3	4.3	4.3
Garlon	L	>10.0	4.2	4.2	4.2
Urab	L	5.6	4.7	4.3	3.8
Urox	L	>5.6	4.0	4.0	4.0
Hyamine 2389	L	2.9	2.4	2.4	2.4
FW-925	L	>10.0	7.5	2.9	2.1
Kuron	L	>10.0	4.0	2.4	2.0
Hyamine 3500	L	>1.0	0.75	0.75	0.75
Penco 191	L	0.75	0.40	0.35	0.35
Copper sulphate	Gr.	>0.8	0.10	0.10	0.10
Hydrothol 47	L	0.29	0.12	0.10	0.08
Phygon XL	Pdr.	0.06	0.04	0.04	0.04
Aqualin	L	0.10	0.04	0.04	-
NIA 5625	L	>0.10	0.07	0.04	0.03
Simazine	Pdr.	Non-toxic up to 18 ppm.			

Differences in fish toxicity due to variations in formulation of a chemical as mentioned earlier, are noteworthy. The toxicity of 2,4-D products increases drastically when the ethyl hexyl esters are replaced by propylene

glycol butyl ether esters. Similarly, silvex becomes considerably more toxic in the butyl ether formulation than it is when used as the potassium salt. The data indicate that cognizance of the complete formulation is requisite if such chemicals are to be used without producing undesirable effects.

Products comprising the last three groups in Table 4 (TL_m's less than 10 ppm.) would be considered potentially lethal to fish since the TL_m's were of the same order to magnitude (or lower) as suggested application rates. In practice, of course, fish may not encounter the applied concentration of chemical due to diffusion, and absorption by vegetation and other organic matter. There is also the possibility of detoxification, where a concentration may be reduced below lethal levels with time. Nevertheless, it is evident that extreme care must be exercised in the choice and application of these chemicals if fish populations are to be maintained.

RESIDUAL TOXICITY TO FISH

The products Kuron, Esteron 99 and Aqualin were tested to determine their toxicity to lake emerald shiners at certain intervals after application. Procedures followed were similar to those for acute toxicity except that, in addition to immediate exposure, fish were exposed to test solutions or granular applications which had been allowed to stand for 6, 24, 48 and 96 hours. With an overall mean length of 8.3 cm., the shiners were somewhat larger than the acute toxicity test stock, necessitating the use of three fish in duplicates of each concentration. Exposure periods were limited to 48 hours.

The 48 hour TL_m's obtained (Table 5) increased with the length of time elapsed after application. The data for Aqualin show an increase from 0.04 ppm. with exposure at 0 hours, to 0.42 ppm. after 96 hours. The TL_m's of Esteron increased likewise from 2.4 to 5.6 ppm., and that for Kuron from 0.71 to 1.0 ppm.

Table 5. Residual toxicity of three herbicides to lake emerald shiners.

Fish exposed at:	48 hour TL_m, ppm. active Kuron	Esteron 99	Aqualin
0 hours	.71	2.40	.04
6 "	.75	2.75	.06
24 "	.75	2.40	.18
48 "	.86	3.20	.40
96 "	1.00	5.60	.42

It will be noted that the TL_m's at 0 hours are somewhat lower than those obtained in the acute toxicity tests. These discrepancies were probably due to differing sensitivities in two fish stocks; they do not negate the comparative value of the data, however.

The data indicate that relatively little detoxification of Kuron took place under the conditions stated. The toxic level of Aqualin decreased ten times after standing 96 hours and that of Esteron, two times. These data should indicate minimal detoxification if degradation is benefited by biological activity. During the course of the acute toxicity tests it was observed that applications of Esteron at 6 and 12 ppm. (acid equiv.) which had killed 100 per cent of the fish in 24 hours were non-toxic to another sample of fish exposed on the second day. Kuron, on the other hand, at 10 ppm. (acid equiv.) killed the second group of fish within 24 hours, as did Aqualin at 1 ppm. active. Evidently, the degradation of 2,4-D was hastened considerably by biological activity. This may have been affecting the other chemicals also, but at slower rates.

FIELD OBSERVATIONS ON THE TOXICITY OF ALGICIDES AND HERBICIDES

When time permitted, observations were made in the field to determine the effects of applications of chemicals on fish, and various types of immature insects and other organisms. These studies were conducted in ponds being treated by personnel of the Metropolitan Toronto and Region Conservation Authority and in five different lakes where experimental applications of Aqualin were undertaken.

FARM POND STUDIES

In four different farm ponds, quantitative assessments of toxicity to invertebrates were provided by retaining in cages organisms which inhabited each pond. These observations were supplemented by qualitative evaluations made by randomly sampling the bottoms of the ponds before and several days after the chemicals were applied. Nymphs of dragonflies (Anisoptera) and damselflies (Zygoptera) were the predominant bottom organisms present in the ponds and these insects were used in the quantitative studies in each situation. Mayflies (Ephemoroptera) were employed in all but one test. Leeches (Hirudinea) were used in two of the four ponds and spotted newts, Triturus viridescens, were collected and employed on one occasion.

Ten to 20 of each of the various types of organisms were collected and placed in cages made from 20-mesh bronze screening, separated so that interspecific predation would not become a problem. Bigger cages made from 8-mesh screening were used for the larger test organisms. Some mortality caused by handling the more fragile forms was anticipated, but with the results achieved in all but one case, this possibility did not materialize to make interpretations difficult. Half of the various kinds of organisms used in each test were

suspended one to two feet below the surface and the other half were lowered to the bottom of the pond.

Surface water temperatures ranged from 65° to 70° F. at the time the chemicals were applied and alkalinity levels in the ponds, expressed as $CaCO_3$, ranged from 64 to 134 ppm. The pH varied from 8.2 to 9.4. Tests indicated that adequate dissolved oxygen was present in the ponds when the post-treatment observations were made.

Table 6, following, illustrates the chemicals used, the organisms tested and the nature of the effects on caged invertebrates in the four farm ponds included in this study.

Table 6. Mortality of organisms held in cages in treated ponds.

	Alkalinity ppm. as $CaCO_3$	Chemical Used	Application Rate	Duration of test	Organisms tested	Number in test	Number Surviving
Pond 1	134	Copper sulphate	1 ppm.	4 days	Mayflies	10	10
					Dragonflies	35	35
					Damselflies	28	20
					L.M. Bass (immature)	20	0
Pond 2	106	Copper sulphate	1 ppm. on 3 consecutive days	5 days	Dragonflies	18	14
					Damselflies	12	11
					Leeches	6	0
Pond 3	64	Aquathol -silvex	2 ppm. (active)	3 days	Dragonflies	15	15
					Damselflies	10	8
					Mayflies	8	6
Pond 4	102	Kurosal "G"	2 ppm. (active)	5 days	Dragonflies	15	15
					Damselflies	15	15
					Leeches	6	6
					Spotted Newts	10	10

In view of the results obtained with damselflies in Pond 2, handling may have been responsible for the eight damselflies dying in Pond 1 which received only one-third as much copper sulphate. The mortality experienced by the tiny young-of-the-year largemouth bass in Pond 1 appeared to be general throughout the pond, as sampling following the treatment showed their numbers to be drastically reduced. This was later substantiated by a thorough seining programme which indicated almost no survival of young-of-the-year bass. No mortality of mature bass resulted from the application.

Pond 2 was treated on three consecutive days at 1 ppm. to provide control of Chara sp. On the day that the third application was made, two speckled trout died. The day following, 70 more speckled trout succumbed and an additional 25 died the next day. It was of interest that the mid-depth dissolved oxygen concentration dropped from supersatuation (23.5 ppm.) on the day prior to treatment which was bright and sunny, to 4.8 ppm. three days after the final application, when the pond was clear of algae.

No mortality resulted among bass inhabiting Pond 3 which was treated with Aquathol-silvex, and the application of Kurosal "G" to Pond 4 produced no evidence of adverse effects to speckled trout.

LAKE STUDIES AND OBSERVATIONS

In addition to the work on farm ponds, tests involving caged fish and invertebrates were conducted during three of five experimental applications of Aqualin to different lakes. These were made in different sorts of physical situations, including open water in the centre of a large bay, an exposed shoreline, a canal, near an entrance to a small bay and to a lake containing much softer water than any of the others. It had been suggested that fish could be herded

away from areas to be treated by careful application techniques since they were reported to exhibit a strong avoidance reaction to this chemical. Since the experimental applications of Aqualin were made on consecutive days, it was possible to make only short-term observations following each application.

Approximately 500 ml. of Aqualin was injected to the windward edge of a $\frac{1}{4}$-acre plot on the exposed shoreline of Rice Lake to disperse any fish. After a few minutes the balance of the chemical was applied to provide a concentration of 4 ppm. No fish were killed by this application other than those held in cages. All of the fish caged within the plot were killed within a four-hour period, including twenty perch, Perca flavescens, two rock bass, Ambloplites rupestris, five spottail shiners, Notropis hudsonius, and four sunfish, Lepomis gibbosus. Most of the perch were in obvious distress after thirty minutes, as were the spottail shiners. There was no mortality among twenty perch and four rock bass retained as a control at a safe distance from the treated area. Damselflies were held both near the surface and at the bottom in the centre of the plot and all 29 of these insects were alive after 9 hours. Three of four crayfish, Cambarus sp., died within the 9-hour period following the application. All of eight planorbid snails used in the test were killed also.

Rapid toxic effects were noted to perch, sunfish, spottail shiners and smallmouth bass, Micropterus dolomieu, caged in an area in Buckhorn Lake in Peterborough County, which was treated with Aqualin at 4 ppm. Only two of 40 fish were alive after $1\frac{1}{2}$ hours, and once again perch and spottail shiners, along with the smallmouth bass, were most susceptible to the chemical since they were all dead after 40 minutes. Damselflies and mayflies were still alive after two hours but all of the snails which were retained succumbed within this period. In spite of an attempt to herd fish out of the area, a sizeable fish kill resulted from this application with sunfish, perch, rock bass, spottail shiners and

smallmouth bass being the principal species affected. A few larger fish which had never come to the surface were observed lying on the bottom. Many tadpoles were killed also.

At Black Lake in Haliburton County, a less productive soft water lake ($CaCO_3$ - 26 ppm.), no significant fish mortality developed in a ¼-acre plot treated with 4 ppm. Aqualin except among those held in cages. After three hours, nine finescale dace, Chrosomus neogaeus, nine perch and fifteen sunfish had died in the cages, while six sunfish were showing signs of distress and six more were behaving normally. Adult sunfish were observed to leave their nests during the treatment. Damselflies, mayflies, crayfish and leeches were the only invertebrates retained in cages and these showed no ill effects after three hours.

Apart from the tests involving caged fish and insects, observations of fish mortality were made when experimental applications were permitted at Frenchman's Bay in Lake Ontario, and in the Trent Canal near Peterborough. Between 100 and 200 sunfish died within a twenty-minute period after Aqualin had been applied at 4 ppm. to an open situation in the middle of Frenchman's Bay. Subsequent reports of larger fish dying as a result of the application were not well substantiated. Treatment of a portion of the Trent Canal adjacent to a lift lock presented a high hazard to fish because of the possibility of fish becoming trapped. Unlike the treatment at Frenchman's Bay, there was no fish mortality apparent within one hour after Aqualin had been applied at 2.5 ppm. to a strip 660' long and 30' wide, and so a second 660 foot-section of the proposed mile-long application was completed. A wait of two hours was then imposed to ensure that fish mortality was not going to become a problem. Near the end of this two-hour period surprising numbers of fish started to appear along both sides of the canal, seemingly trying to get as far away as possible from the strip which had been treated in the centre. Erratic swimming and unusual surfacing movements were exhibited and large numbers

of fish, most of them of small size, began to die. Species involved were sunfish, rock bass, smallmouth bass (fry), white suckers and carp. One dead yellow pickerel, Stizostedion vitreum, was also collected. Subsequent reports of fish mortality below the lift lock were received even though only a small volume of water was by-passing the lift lock structure.

Conclusions

Single applications of 1 ppm. copper sulphate should not kill mature bass or immature aquatic insects, but may be lethal to young-of-the-year bass.

Applications of 1 ppm. copper sulphate on three consecutive days will kill leeches and will endanger speckled trout populations, but will not adversely affect mayfly and dragonfly nymphs.

Aquathol-silvex and Kurosal "G" applied at 2 ppm. are not acutely toxic to fishes, leeches, newts or immature insects.

Aqualin at 4 - 5 ppm. causes mortality among a variety of fish species. The contention that this herbicide can be applied so that fish will leave and avoid an area of treatment cannot be relied upon, according to observations made during these tests. Fish mortality associated with the use of this chemical may be negligible or extensive and may be spontaneous or may not develop until one or two hours have passed. The degree and rapidity of mortality probably depends largely on the size and shape of the area to be treated and physical conditions present. Aqualin is not as acutely toxic to invertebrates as it is to fish, except that planorbid snails succumb rather quickly. It should be emphasized that only short-term observations could be made following the applications of Aqualin, with a maximum observation period of nine hours for the caged invertebrates.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Data obtained on the intensity of taste and odour of herbicides in water indicate that application of endothal and the potassium salt of silvex (Kurosal) should not present a problem in this respect. The adequacy of dilution must be considered in applying Aqualin, Hydrothol 47, Kuron and 2,4-D formulations in order to negate the possibility of taste and odour in water supplies.

A wide range of acute toxicity to native fish exists among the various products available. Several afford a considerable margin of safety for fish and selection of one of these should be made whenever applicable to the problem. Certain other chemicals are moderately toxic but provide an adequate safety margin when used at rates suggested for weed control. There are several preparations, however, which are toxic to fish at concentrations of the same magnitude, or lower, as are commonly applied. Although fish may not encounter the applied concentration, use of these chemicals should be limited and judicious.

Field observations on applications of chemicals supported laboratory results indicating that Aquathol-silvex and Kurosal G could be used without harm to fish and, in addition, to invertebrate life. Copper sulphate may be used safely at 1 ppm. in farm ponds where young-of-the-year fish are not involved. Three applications of 1 ppm. copper sulphate on consecutive days should not endanger immature insects but may be lethal to speckled trout. Aqualin is not a safe herbicide to use where fish populations must be protected but it appears to be less acutely toxic to invertebrate life. Permits should not be issued under the Ontario Water Resources Commission Act to authorize the use of Aqualin for aquatic weed control in public waters.

Fish toxicity of aquatic herbicides may decrease with time, the rate depending on the chemical involved. The detoxification of certain products is hastened by biological activity.

In selecting a herbicide cognizance must be taken of different formulations of a chemical, as the formulation influences fish toxicity and intensity of taste and odour in water.

REFERENCE

A.P.H.A., A.W.W.A., W.P.C.F., 1960. Standard methods for the examination of water and wastewater. 11th ed., xxi + 626 pp., A.P.H.A., New York.

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